

I HEREBY CERTIFY THAT THE CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST CLASS MAIL IN ENVELOPE ADDRESSED TO: COMMISSIONER FOR PATENTS, P.O. BOX 1450, ALEXANDRIA, VA 22313-1450, ON THE DATE INDICATED BELOW.

By: Helene Gabel

Date: September 29, 2005

MAIL STOP AMENDMENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Patent Application of: :
Ye Wu *et al.* :
Conf. No.: 4224 : Group Art Unit: 1614
Appln. No.: 10/627,483 : Examiner: Thomas C. McKenzie, Ph.D.
Filing Date: July 25, 2003 : Attorney Docket No.: 066131-53U1
: (X-0219)

Title: PROCESS FOR SYNTHESIZING ANTIFOLATES

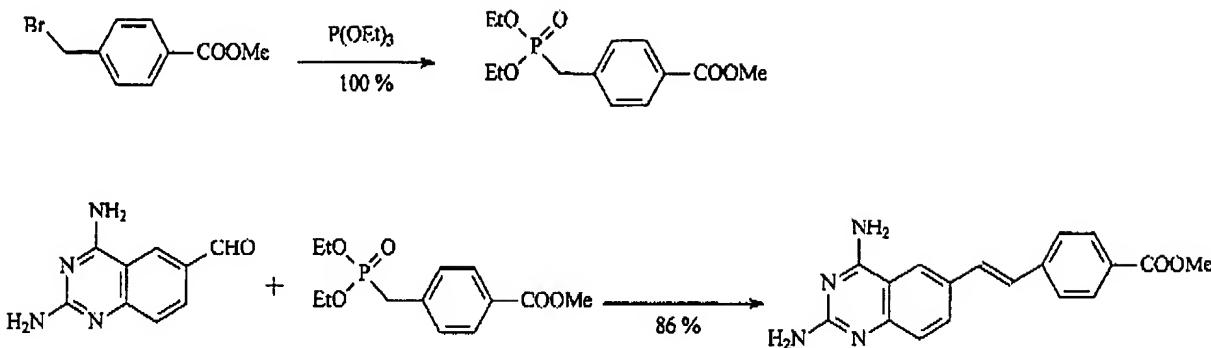
SUPPLEMENTAL DECLARATION OF HARRY KOCHAT, PH.D.
UNDER 37 C.F.R. § 1.132

I, Harry Kochat, Ph.D., declare as follows:

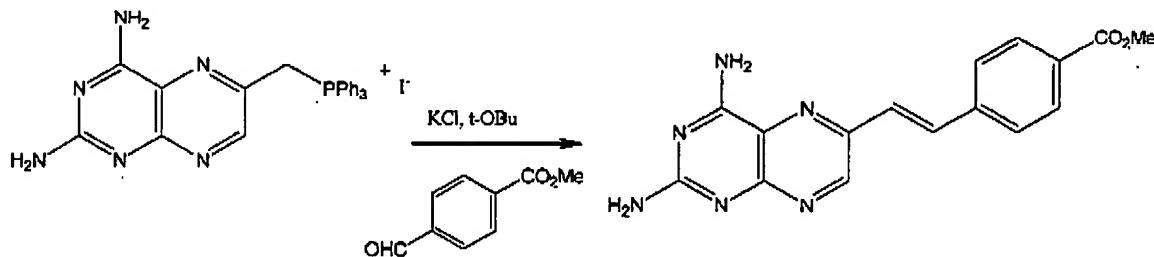
1. I am the same Harry Kochat who is named as a coinventor in the above-identified patent application and who executed a Declaration on June 15, 2005 (hereinafter "my first Rule 132 Declaration"), filed with the U.S. Patent and Trademark Office on June 23, 2005.

2. The following pair of reactions were performed as set forth in Examples 5 and 6 of the application, with a yield of 86%, as reported in my first Rule 132 Declaration. The second of the reactions shown in this paragraph uses what was referred to in my first Rule 132 Declaration as a "modified Wittig reaction" and referred to as a "modified Horner reaction" at page 11, line 21, of the application, (hereinafter, a modified "Wittig-Horner reaction" to avoid confusion), which is capable of being used in a large-scale manufacturing process for the unsaturated precursor for and ultimately, the exemplary product covered at least by claim 3 of the application.

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3. The following reaction, using the procedure below, was performed for BioNumerik Pharmaceuticals, Inc., the assignee of this application and invention (hereinafter "BioNumerik"), by one of BioNumerik's contract laboratories, to produce the same product as in the second reaction set forth in paragraph 2 above, but using the standard Wittig reaction:



Procedure:

2,4-Diamino-6-(chloromethyl)pteridine hydrochloride (2.47 Kg, 10 mol) and 33% w/w dimethylamine in ethanol (0.27 Kg, 2.0 mol) were taken up in dimethyl formamide (67.5 Kg, 71 L) and stirred at ambient temperature for 30 minutes. Potassium iodide (5.32 Kg, 32 mol) was added and stirring continued for an additional hour to convert the substrate into triphenylphosphinomethyl pteridinium iodide. The above mixture was then cooled to -10 °C and methyl 4-formylbenzoate (2.14 Kg, 13.0 mol) was added. After being stirred for 10 minutes, a solution of potassium t-butoxide (2.82 Kg, 25.1 mol), a strong organic base, was added over a period of 1 hour at -10°C. The suspension was heated to 55 to 60 °C for 30 minutes. The mixture was then cooled to room temperature and centrifuged to obtain the

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product. The filter cake was washed with dimethylformamide (13.3 Kg, 14 L) and subsequently with methanol (16.6 Kg, 21 L). The wet product (6 to 10 Kg) was sampled for in-process analysis. The above product was contaminated with triphenyl phosphine oxide by-product. Since it was not possible to purify the product from the above by-product, the crude product was taken into the subsequent hydrogenation reduction step as shown in Example 7 of this patent application. The estimated content of the product from HPLC was 2.32 Kg (72.8 % yield by peak area).

4. At BioNumerik, we have consistently observed a low yield in the case of Wittig reactions performed not only on the compound shown in the second reaction in paragraph 2 and in the reaction shown in paragraph 3 above, but also on various compounds besides those within formula Ib of the application or their unsaturated precursors, irrespective of the functional group present on the phenyl rings. Thus, the yields for products where R₄ of formula Ib or their unsaturated precursors are γ -methylene glutamic acid, glutamic acid, aspartic acid or γ -methylene glutamate residues, as claimed in claim 3, also would be expected to be low if the Wittig reaction were used, but now, after the present invention, the yields of such compounds would be expected to be improved if the modified Wittig-Horner reaction of the invention were used. As a result of the low yields prior to the present invention, we sought a better process to make such compounds, including compounds within formula Ib of this application, or their unsaturated precursors. This research led to the present invention involving the modified Wittig-Horner reaction, but it was not easy to do, despite the statement in the Advisory Action of July 20, 2005, about the "notorious" problems of triphenyl phosphine oxide "for being bulky, difficult to remove and for appearing in every chromatographic fraction."

5. Even assuming only for the sake of argument that the problems were well known, their solutions were not, particularly with respect to making a compound within formula Ib or its unsaturated precursor. First, our claimed process was the first to ever use the modified Wittig-Horner reaction on a pteridine moiety or a quinazoline moiety, following research that did not reveal any reports of such use. Even BioNumerik's contract manufacturers did not suggest this process. Second, the unprotected amino groups on the pteridine compound provide points of reaction or attack that could prevent a coupling reaction of the pteridine compound to produce

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the unsaturated intermediate shown in Example 6 of the application and in the reactions of paragraphs 2 and 3 above. These interfering reactions are typically avoided by using a base such as the potassium t-butoxide used in the Wittig reaction in paragraph 2 above, and it was expected that an organic base would have to be used in the modified Wittig-Horner reaction. It was surprising that by using the phosphonate reactant of the modified Wittig-Horner reaction, the phosphonate reactant has a mild basicity that allowed the coupling reaction to proceed without using a separate organic base.

6. The current invention is an improved process that is suitable for scale-up efforts. In process improvement and scale-up programs, chromatographic isolation as used in the House reference is not practical, due to a huge amount of chromatographic adsorbent or solid support that would be needed, together with a huge amount of solvent that would be needed to elute the product. In all scalable processes, because of these issues, chromatographic isolation has to be substituted with crystallization or slurring techniques, where the compound to be isolated is not yet suitable for crystallization, due to thermal instability. Triphenyl phosphine oxide, the by-product generated during the Wittig reaction, is practically impossible to separate from the product via crystallization or slurring. By inventing the chemical transformation described in the invention, we have conceived of and reduced to practice the modified Wittig-Horner reaction with the claimed compounds, instead of the standard Wittig reaction, whereby chromatographic separation is completely eliminated. Using the modified Wittig-Horner reaction that we invented with the claimed compounds, we could eliminate the formation of the highly insoluble triphenyl phosphine by-product. The by-product salt in the corresponding modified Wittig-Horner reaction is triethyl phosphate, which is highly water soluble. Thus, extraction of the organic portion with water could remove such by-product conveniently in all scaled-up processes. This process was not obvious to us, because of the lack of any disclosure anywhere, including the House reference cited in the prosecution of this application, of the successful use of a modified Wittig-Horner reaction involving pteridine or quinazoline compounds as claimed for the reasons discussed above. For the same reason, it is my strong opinion that our invention would not have been obvious to a person of ordinary skill in the art, in view of the issues that we faced, again as described above.

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7. The Advisory Action included the statement that "an increase in the yield from 73% to 83% [sic] is a change in degree not in kind." Contrary to the implications of this statement, from a commercial manufacturing perspective, even such a 10% increase in yield, let alone the actual increase of 13% to an 86% yield (rather than 83% in the quoted language from the Advisory Action), is very important, not only with respect to economy relating to the chemicals involved, but also with respect to the lack of necessity of disposing of unwanted byproducts, and the time and labor involved. More importantly, due to the contamination of the intended product, the intended product was simply not usable, rendering the process involved in the Wittig reaction meaningless.

I hereby declare that all statements made herein of my own knowledge are true and that all statements on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application or any patent issuing thereon.

Date: September 27, 05

Harry Kochat

Harry Kochat, Ph.D.